Listeria monocytogenes EGD chitinolytic activity is regulated by carbohydrates but also by the virulence regulator PrfA

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REFERENCE A/P 15

Listeria monocytogenes EGD chitinolytic activity is regulated by carbohydrates but also by the virulence regulatory gene, PrfA

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Chitin, a highly insoluble polymer of N-acetyl-D-glucosamine (GlcNAc), is a major component of fungal cell walls and insect and crustacean exoskeletons and is one of the most abundant polymers in marine and terrestrial environments. Chitin hydrolysis by *Listeria monocytogenes* depends on two chitinase encoding genes *chiA* (*lmo1883*) and *chiB* (*lmo105*) and the aim of this study was to investigate their regulation. The production of the chitinases is substrate regulated and subjected to catabolite control. Thus, chitin induces expression of both chitinases however *chiA* but not *chiB* is furthermore induced by the monomer GlcNAc. In growth medium supplemented with chitin and glucose the expression of both chitinases is repressed. Expression of *chiA* and *chiB* is growth phase dependent with higher expression in late exponential growth phase compared to early exponential phase. Chitinases expressed by bacterial pathogens have proven to be important not only for nutrient acquisition and environmental survival but also for infecting humans and animals. Interestingly, we found that the central *L. monocytogenes* virulence gene regulator, PrfA, is required for the chitinolytic phenotype as chitinase activity was significantly reduced in Δ*prfA* mutant cells compared to the wild type cells. In agreement with this result northern blot analysis showed that the amounts of *chiA* and *chiB* transcripts were significantly lower upon induction by chitin in the Δ*prfA* mutant compared with the wild type. Furthermore, in contrast to the wild type, no *chiA* transcript could be detected in the mutant lacking PrfA during growth in medium supplemented with GlcNAc. Regulation of chitinolytic activity in *L. monocytogenes* is complex and the results obtained in this and other studies indicate that the biological role of this activity may not be limited to the external environment.

REFERENCE A/P 16

Infectious dose curves for guinea pigs challenged with a *Listeria monocytogenes* epidemic clone strain and a strain carrying a naturally-occurring virulence-attenuating mutation in *inlA* show a significant shift in median infectious dose

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Listeria monocytogenes contains two subpopulations, including (i) epidemic clone (EC) strains, which have been linked to the majority of listeriosis outbreaks worldwide and are overrepresented among sporadic cases in some countries along with (ii) strains carrying mutations leading to a premature stop codon (PMSC) in *inlA*, which are commonly isolated from ready-to-eat foods (approx. 45%) but rarely associated with human disease (approx. 5%). The virulence factor Internalin-A (InlA; encoded by *inlA*) binds certain isoforms of the cellular receptor E-cadherin to facilitate crossing of the intestinal barrier by *L. monocytogenes*. The current FDA/FSIS/CDC *L. monocytogenes* risk assessment was developed with dose response data from murine challenge experiments, a model that fails to probe InlA mediated virulence due to the inability of InlA to bind the murine isoform of E-cadherin. Guinea pigs, which express the human isoform of E-cadherin that binds InlA, were intragastrically challenged with (i) a fully-invasive EC strain associated with a listeriosis outbreak or (ii) a strain carrying the most common *inlA* PMSC mutation. Dose-response curves for tissue infectivity were constructed with either a log-logistic, beta poisson, or exponential (for spleen data) fit to the raw individual and combined organ data. The log logistic and beta poisson models based on combined organ data showed an approx. 1.3 log10 CFU increase in the median infectious dose for the strain carrying a PMSC in *inlA* relative to the EC strain. Inclusion of strain effect significantly improved the ability of the model to explain the observed data, supporting a significant difference in tissue infectivity between EC and PMSC strains. *L. monocytogenes* strains thus show notable differences in infectious dose required to establish an infection. Results from this work support the dose-response relationship for *L. monocytogenes* is strain-specific and will provide critical data for enhancement of existing and development of future risk assessments.